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**14. ABSTRACT**

This project studies the feasibility of using solid-liquid phase change nanoparticles to detect multiple cancer biomarkers for enhanced cancer detection at early stage. With the support from DOD-LCRP, we have proved the new signal transduction mechanism based on solid-liquid phase change nanoparticles works for the detection of multiple proteins. A series of metal and alloy nanoparticles have been made and used for the detection of multiple proteins. The melting peaks are used for qualitative and quantitative detection of biomarkers. This grant supported a graduate student and a postdoc. This project has produced ~10 peer-reviewed publications, and the result has been used to obtain an NSF-CAREER project in 2011, as well as two grants to NIH and National Institute of Justice in 2012.

**15. SUBJECT TERMS**
Cancer early detection, molecular biomarkers, phase change nanoparticles, multiplicity

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**Annual report**

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**1. Building foundation of thermal biosensing**

Nanoparticles of metal or eutectic alloys with sharp melting peak can be modified with antibodies at certain grafting density. A high thermal conductivity substrate (silicon, or aluminum) will be modified with multiple antibodies. Nanoparticles will be immersed in a buffer containing biomarkers; after incubating for certain time, the substrate will be put into the solution to capture nanoparticles by forming sandwich type complexes. After washing to remove excess particles, the nanoparticles immobilized onto the substrate will be detected by using differential scanning calorimetry (DSC), where the peak position and peak area reflect the presence and concentration of biomarkers. A series of metallic nanoparticles with composition-encoded melting points will be utilized to detect multiple antigens after creating a one-to-one correspondence between one type of nanoparticle and one type of biomarker. The multiple detection result will be used to detect lethal cancer by statistical analysis.

*High multiplicity:* By using a commercial DSC, the peak widths at half maximum of metal nanoparticles or eutectic alloy nanoparticles can be 0.6°C at ramp rate of 1°C/minute. If thermal scan range is from 100 to 700°C, the maximal number of melting peaks that can be resolved will be 1,000 based on Rayleigh's criterion on spectral resolution which means that 1,000 different types of cancer biomarkers could be detected in a thermal scan by detecting nanoparticles.

*Eutectic nanoparticles:* Although metal nanoparticles have sharp melting peaks, there are a limited number of metals in the periodic table to produce nanoparticles due to availability and safety issues. Alloy nanoparticles of eutectic composition have one sharp melting peak, and also composition dependent melting temperatures (providing sizes are larger than 10 nm). According to combination rule, if any two among three metals can form binary eutectic alloys, the three metals can form one ternary eutectic alloy. The total number of metals and eutectic alloys is seven (Figure. 1). For a given number of metals that form binary eutectic alloys among any two of them, the numbers of binary alloy, ternary alloy and so on can be derived from Pascal's triangle, and the total number of metals and eutectic alloys can be derived from $n$ (the number of metals) and $k$ (the number of metals in particle) as

$$\sum_{k=1}^{n} C_n^k - 1 = \sum_{k=1}^{n} \frac{n!}{k!(n-k)!} - 1 = 2^n - 1$$

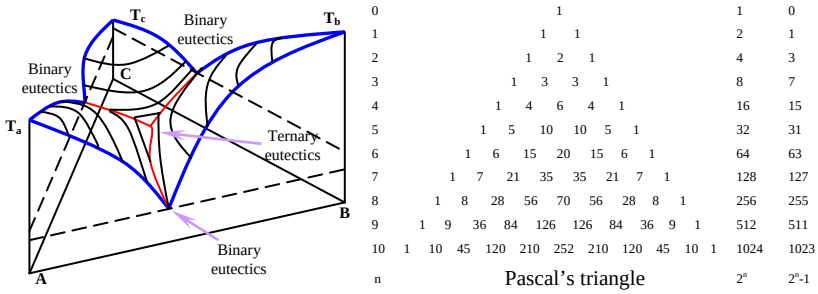


**Figure 1.** Ternary phase diagram, and Pascal's triangle.

*Detection limit:* Assuming that nanoparticles have the same size, the sensitivity can be enhanced by reducing the grafting density of ligand around nanoparticles. Taking the root mean square (RMS) noise of a DSC instrument as 0.2 μW, the minimal detectable heat flow will be 0.2 μJ for a 1°C wide peak at ramp rate of 1°C/second. If 30 nm diameter copper nanoparticles (latent heat of 205 J/g, density of 13.6 g/cm$^3$) were used, the number of nanoparticles that absorb 0.2 μJ heat in phase change is $2.6\times10^6$. Providing that one antibody is attached on each nanoparticle, the according antigen concentration in 1 ml solution is $4.3\times10^{-15}$ M or 4.3 fM, which is lower than existing nanoparticle techniques. By using nanoparticles of large diameter, or materials with large latent heat, the detection sensitivity can be further increased.

*Concentration range:* The concentration range of thermal detection can be controlled to detect low and high concentration biomarkers under the same condition at the same time. Few parameters can be changed to adjust concentration detection range: (1) nanoparticle size, (2) latent heat of fusion, (3) ligand grafting density, (3) nanostructured surface, (4) operating condition, and (5) ligand grafting density. By changing particle size from 10 to 200 nm, the measured heat flow can be adjusted over 3 orders of magnitude; by changing material, the heat flow can be adjusted over 1 order of magnitude (latent heat

of fusion of copper is 200 J/g, and that of indium is 20 J/g); by using nanostructured substrate, the surface area can be 2 orders of magnitude larger; by changing ramp rate, the heat flow changes over 3 orders of magnitude (variable ramp rate can be achieved in the same thermal scan); by changing ligand grafting density, the detectable concentration of biomarker can be adjusted over 2 orders of magnitude. As each parameter can be changed independently, the concentration range will be 11 orders of magnitude in ideal situation.

*Uniqueness:* Although enzyme thermistor can detect heat generation related to analyte, the multiplicity is low: only one species can be detected each time. Nanoparticles of low melting point metals and alloys have been made as solder materials or heat transfer additives, but there is no attempt for biomarker detection due to lack of awareness on their unique thermal properties, and critical need to detect multiple cancer biomarkers.

## 2. Experimental results

10 metals have been identified that form binary eutectic alloy among any two of them: aluminum, bismuth, cadmium, copper, gadillium, indium, lead, magnesium, palladium and silver. These metals can make 10 types of metal nanoparticles, 45 type of binary alloy nanoparticles, 120 types of ternary eutectic alloy nanoparticles, 210 types of quaternary eutectic alloy nano-particles, and so on. The total number of metals and eutectic alloys is 1,023. The nanoparticles of metals and eutectic alloys have sharp and discrete melting peaks that can be resolved by using DSC with high resolution (0.01°C). We had made nanoparticles of low melting point metals (tin, indium and bismuth) and eutectic alloy (lead-tin alloy) by decomposing organometallic precursors at stoichiometric ratio. Figure 2A shows a scanning electron microscopy (SEM) image of bismuth nanoparticles (diameter 200 nm). By changing molar ratio of surfactant and precursor, 30 nm diameter nanoparticles have been made as shown in transmission electron microscopy (TEM) image (Figure 2B). The melting point of bismuth nanoparticles is the same as bulk bismuth (271°C), and the peak area in DSC is proportional to the mass of nanoparticles.

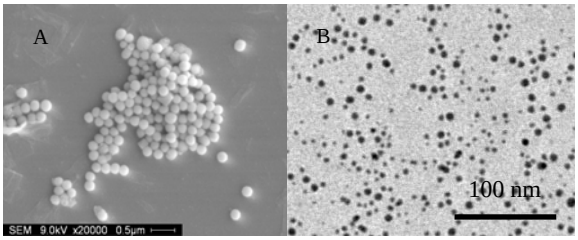


**Figure 2.** SEM (A), and TEM (B) images of bismuth nanoparticles.

The surfaces of nanoparticles have been modified as below: (1) thin oxide films are made at nanoparticles by heating nanoparticles at 120°C in air for 10 min; (2) oxide films are modified with aminopropyltriethoxysilane (APTES); (3) the amine groups at nanoparticles are conjugated with avidin using disuccinimidyl suferate as crosslinkers. X-ray photoelectron spectroscopy (XPS) spectrum collected during argon ion sputtering shows signals of metal and oxide (Figure 3A); while XPS spectrum collected before sputtering shows only oxide signal. DSC is used to detect nanoparticles after immobilization onto an aluminum substrate *via* biotin-avidin interaction (Figure 3B), where melting peak at 157°C is from indium nanoparticle. As biotin concentrations change from 20, 2 to 0.05 ng/ml, the heat flow reduces. The results indicate that: oxide films are formed at nanoparticles; nanoparticles have metallic cores; biomolecules have been conjugated onto nanoparticles using DSS crosslinker.

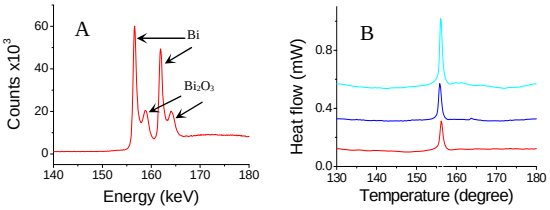


**Figure 3.** XPS spectrum of surface oxidized bismuth nanoparticles (A); DSC curves of indium nanoparticles immobilized on aluminum by biotin-avidin interaction (B).

The sensitivity, multiplicity, and analysis time are determined by $\Delta H$, $w$, and $\beta$, respectively, and are related to each other. The width of melting peak can be derived from

$$w = RC_s[\sqrt{1+\frac{2\Delta H}{RC_s{}^2\cdot\beta-1}}+\ln 100]\cdot\beta$$

where $R$ is thermal resistance, $C_s$ is heat capacity of sample, $\beta$ is ramp rate, and $\Delta H$ is fusion enthalpy. Figure 4 shows the relation between ramp rate and peak width, where the peak width is less than 1°C at small ramp rate.

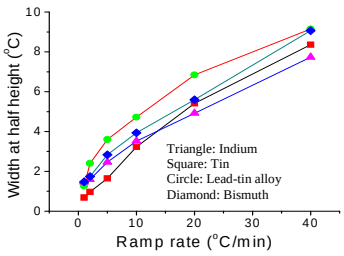


**Figure 4.** Temperature ramp rate dependent peak width of nanoparticles.

We have successfully detected four distinct proteins using phase change nanoparticles (tin, tin-lead alloy, indium, and bismuth). Figure 5A shows the DSC curves of indium nanoparticles attached onto aluminum plates after detecting rabbit IgG in buffers, where IgG concentrations are 200, 20, 2, and 0.5 ng/ml, respectively. The detection limit is

close to that of other nanoparticle-based techniques. These four types of nanoparticles have been used to detect rabbit IgG, human IgG, prostate specific antigen (PSA) and biotin. Figure 5B are melting peaks of four nanoparticles. Since the nanoparticles made in this work melt at the same temperature as bulk materials, the possible cross-talk among nanoparticles during heating process is excluded.

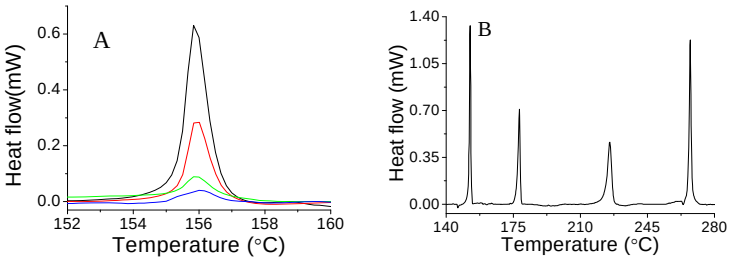


**Figure 5.** Thermal detections of rabbit-IgG in buffers using indium nanoparticles (A); simultaneously detection of four proteins (rabbit IgG, human IgG, biotin, and PSA);

## 3. Related publications


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2. J. J. Hu, C. Muratore, J. G. Jones, A. A. Voevodin, Y. Hong, M. Su, In situ transmission electron microscopy of phase change investigation of bismuth nanoparticles, *Nanoscale* 2011, 3, 3700.
3. C. Wang, L. Ma, M. Su, Simultaneous detection of multiple biomarkers with several orders of concentration difference using phase change nanoparticles, *Anal. Chem.* 2011, 83, 2215.
4. M. Hossain, C. Wang, M. Su, Multiplexed biomarker detection using X-ray fluorescence of composition-encoded nanoparticles, *Appl. Phys. Lett.* 2010, 97, 263704.
5. C. Wang, M. Hossain, L. Ma, M. Su, Highly sensitive thermal detection of thrombin using aptamer-functionalized phase change nanoparticles, *Biosensors Bioelectronics* 2010, 26, 437.
6. C. Wang, L. Ma, L. Chen, K. X. Chai, M. Su, Scanning calorimetric detections of multiple DNA biomarkers contained in complex fluids using phase change nanoparticles, *Anal. Chem.* 2010, 82, 1838.
7. L. Ma, C. Wang, Y. Hong, M. Zhang, M. Su, Thermally addressed immunosorbent assay (TAISA) using encapsulated phase change nanoparticles, *Anal. Chem.* 2010, 82, 1186.
8. M. Su, L. Ma, Y. Hong, Thermo-probes, methods of making thermo-probes, and methods of detection using thermo-probes, US Patent, No. 61 303 396, 2010.
9. M. Su, Multiplexed biosensing with phase change nanoparticles (invited review), Nanomedicine 2013, 8, 1-11.
10. B. Duong, M. Su, Drug anti-counterfeiting with phase change nanoparticles of organic solids, Adv. Mater. 2013, submitted.
11. H. Liu, B. Duong, C. Wang, M. Su, Printable microtaggants with phase change nanoparticles for enhanced security, Appl. Mater. Interface 2013, submitted.


## 4. Related grant

M. Su (PI), CAREER: Biosensing in thermal space (1055599), National Science Foundation, Biosensing Program, 08/01/2011-07/31/2016. $400,000.00.
M. Su (PI), Encapsulated phase change nanoparticles as thermally readable covert taggants (2012-DN-BX-K021), National Institute of Justice, 01/01/2013-12/31/2014. $350,200.00.
M. Su (PI), Enhanced radiation therapy with nanoscale frequency modulators (1DP2EB016572), National Institute of Health, 10/01/2012-09/31/2017. $2,175,000.00.

## 5. Proposal submissions

Based on the preliminary results obtain from this grant, I had submitted proposals to National Institute of Health, Alzheimer's Association and National Institute of Justices.

## 6. Future direction

I will submit NIH SBIR proposal to commercialize this method. In the next few years, I will try to put thermal detection into next level for high throughput multiplexed assay. This may generate a revolutionary technique that has the potential to supplement or even replace existing microarray technology.